

# Interaction between Smoking and Asbestos in Human Lung Adenocarcinoma: Role of *K-ras* Mutations

by Harri Vainio,<sup>1</sup> Kirsti Husgafvel-Pursiainen,<sup>3</sup> Sisko Anttila,<sup>3</sup> Antti Karjalainen,<sup>3</sup> Peter Hackman,<sup>3</sup> and Timo Partanen<sup>3</sup>

To investigate the role of tobacco smoking and asbestos fibers in the etiology of human lung cancer, we examined the activating point mutations in the *K-ras* oncogene in DNA samples from 49 patients. Mutations were found more often in tissue from adenocarcinomas (12/21) than in tissue from tumors other than nonadenocarcinomas of the lung (3/28). Among the adenocarcinoma patients, asbestos exposure was predictive of *K-ras* mutation (odds ratio, 4.9; 95% confidence interval, 0.7-34.3); in patients with other types of lung cancer, the relation appeared to be an inverse one, but the numbers were small. The proportion of heavy smokers (over 50 pack-years) was 60% among people with *K-ras* mutations and 35% among the *K-ras*-negative subjects, suggesting that smoking causes *K-ras* mutations. If mutations in *K-ras* genes are caused by smoking, asbestos would act as a promoting agent by conferring selective growth conditions for clonal expansion on these mutated cells. Asbestos may favor recruitment of (initiated) *K-ras* mutation-positive cells in the multistage process of carcinogenesis by stimulating cellular growth.

## Introduction

Lung cancer is the commonest fatal neoplastic disease in the world. Most human lung cancers can be classified into one of four major types: small cell carcinoma, squamous cell carcinoma, adenocarcinoma, and large cell carcinoma. Tobacco smoking causes all types of lung cancer, but the risk for squamous cell and small cell carcinomas of the lung is increased by cigarette smoking to a greater extent than the risk for adenocarcinoma of the lung (1).

An increased risk for lung cancer has been demonstrated in studies of populations with occupational exposure to asbestos fibers (2). Peripheral tumors (adenocarcinomas) appear to be more closely associated with exposure to asbestos than other histological types of lung cancer (3). The increase in risk for lung cancer occurs in both smokers and nonsmokers, but because there is an

important interaction between exposure to asbestos and smoking, very few cases of lung cancer occur in nonsmoking asbestos-exposed workers (4).

Carcinogenesis is a multistage process in which normal growth, differentiation and development have gone awry. Although it is clear that no single gene can account for all the changes in the multistage progression from normalcy to malignancy, certain oncogenes have emerged as being particularly important. The prototypical example of such oncogenes is *ras* (5), and it is generally conceded that *ras* represents a nexus for the control of cellular proliferation (6,7). *K-ras* mutations are very rare among nonsmokers, and it has therefore been assumed that carcinogens in tobacco smoke cause the mutations directly (6-8).

The mode of carcinogenic action of asbestos is not known; a number of potential mechanisms at the molecular and cellular levels have, however, been proposed (9). Increased serum levels of *ras* oncogene-related protein (P21) were found in 7 of 18 patients with advanced asbestosis who developed cancer (5 cases of lung cancer, 2 of pleural mesotheliomas) and in only 2 of 28 patients without cancer (10). Also, people with asbestosis had elevated serum levels of growth factors, indicating that heavy exposure to asbestos can stimulate cell growth.

Adenocarcinoma is the histological type of lung cancer that is clearly associated with exposure to asbestos, and it

<sup>1</sup>International Agency for Research on Cancer, 150 cours Albert-Thomas, 69372 Lyon Cedex 08, France.

<sup>2</sup>Institute of Occupational Health, Topeliuksenkatu 41 a A, 00250 Helsinki, Finland.

Address reprint requests to H. Vainio, International Agency for Research on Cancer, 150 cours Albert-Thomas, 69372 Lyon Cedex 08, France.

Table 1. Characteristics of lung cancer patients by histological subtype.

Histological subtype	No. of cases	Sex (M/F)	Age, AM (SD)	Pack-years, AM (SD)
Squamous cell carcinoma <sup>b</sup>	22	20/2	63 (8.8)	42 (22)
Adenocarcinoma <sup>b</sup>	21	18/3	60 (10)	42 (29)
Small cell carcinoma	4	3/1	63 (1.8)	30 (24)
Large cell carcinoma	2	2/0	72 (1.4)	62 (38)
All types combined	49	44/6	62 (9.1)	42 (26)

AM, arithmetic mean.

<sup>b</sup>No data on smoking were available for one patient.Table 2. Odds ratio for adenocarcinoma associated with high exposure to asbestos and point mutations in codons 12 or 13 of K-ras.<sup>a</sup>

Predictor	No. of exposed cases of adenocarcinomas	Odds ratio	95% CI <sup>b</sup>
Asbestos fibers ≥3 × 10 <sup>6</sup> /g dried lung	9	3.45	0.94–12.6
K-ras positivity	12	11.1	2.54–45.3

<sup>a</sup>Logit model.<sup>b</sup>95% Confidence interval.

is the type in which smoking plays a lesser role, in comparison to squamous cell cancer. Therefore, the interaction between exposure to asbestos and smoking might be particularly strong in inducing adenocarcinoma. We considered, therefore, that this histological type would be a good model for studying the potential mechanisms of interaction.

In the present study, we examined the role of mutations in one oncogene, the K-ras gene, in the causation of lung cancer and its potential role in the synergistic interaction between asbestos and smoking at the molecular level, especially in cases of lung adenocarcinoma.

## Materials and Methods

Lung carcinoma specimens were obtained during thoracotomy at the Helsinki University Hospital. Of 49 patients, 21 had adenocarcinomas and the remainder had mainly squamous-cell carcinomas (Table 1). DNA was isolated from lung tumor samples by standard procedures. Polymerase chain reaction amplification of the sequences studied in the oligohybridization assay was performed as described by Husgafvel-Pursiainen et al. (11). Asbestos fibers were determined in the lung tissues by transmission electron microscopy, as described elsewhere (12). The data were analyzed statistically by logistic regression (case-referent type) and tabular analysis (cohort type).

## Results

Point mutational activation of the K-ras oncogene was studied in a set of DNA samples extracted from 49 lung tumors. The distribution of tumor histology, with other characteristics of the patients, is presented in Table 1.

Nine (43%) of the 21 adenocarcinoma cases and 5 (17%) of the 28 nonadenomatous lung cancer cases had an asbestos fiber concentration in the lungs that exceeded 3 × 10<sup>6</sup> fibers/g of dried lung, indicating occupational

asbestos exposure (12). This category of heavy asbestos exposure was associated with an odds ratio for adenocarcinoma of 3.5 [95% confidence interval (CI), 0.9–12.6; Table 2]. Twelve (57%) of the adenocarcinoma cases and 3 (11%) of the nonadenomatous lung cancers were K-ras-positive, the odds ratio for adenocarcinoma in K-ras-positives being significantly elevated (11.1; 95% CI, 2.54–45.3; Table 2).

Among the adenocarcinoma cases, asbestos exposure was predictive of K-ras mutation, though not significantly so (odds ratio, 4.9; 95% CI, 0.7–34.3; Table 2). In cancers of other cell types, the relation appeared to be inverse, but the numbers were small (Table 3).

Another interpretation of the interrelations between asbestos exposure, K-ras mutation, and histological type of cancer is presented in Table 4. The risk for adenocarcinoma relative to other cell types is expressed as the odds ratio for adenocarcinoma associated with different combinations of asbestos exposure and point mutations in codons 12 or 13 of K-ras, with the no mutation–low asbestos exposure combination as the reference category for the remaining three combinations. The highest odds ratio (41.0; 95% CI, 2.1–809) was observed for patients with heavy asbestos exposure and K-ras mutation. The odds ratio for those with lower fiber content and K-ras mutation was also elevated but to a considerably lesser degree, not quite reaching statistical significance (odds ratio, 4.8; 95% CI, 0.9–25.3). The odds ratio for patients with high asbestos exposure but no K-ras mutation was near unity.

The proportion of all smokers who were heavy smokers (over 50 pack-years of cigarettes) was 60% among people with K-ras mutations and 35% among K-ras-negative subjects. The corresponding odds ratio was 2.8 (nonsignificant; Table 5).

## Discussion

The interaction between tobacco smoking and exposure to asbestos in the causation of lung cancer is a highly debated issue [see reviews by Steenland and Thun (13) and Saracci (4)]. The interaction pattern is somewhat variable; although the results of epidemiological studies could be reconciled with a multiplicative model, this variation in the strength of the interaction may reflect real differences based on the fact that asbestos and smoking probably act at different stages of the carcinogenic process. Tobacco smoking can obviously act at an early stage: tobacco smoke contains a number of DNA-damaging agents such as polynuclear aromatic hydrocarbons (1). Because it has been shown that asbestos and smoking have a multiplicative effect in causing lung cancer, it may be that relatively

**Table 3. Distribution of (number of subjects) and odds ratios for point mutations in codons 12 or 13 of *K-ras* in adenocarcinoma patients, according to asbestos fiber content in the lung tissue.**

Asbestos fiber content, per g dried lung	K-ras mutation <sup>b</sup>				Odds ratio <sup>a</sup> (M + F)	95% CI <sup>a,b</sup> (M + F)
	Positive		Negative			
	M + F	M	M + F	M		
Adenocarcinoma						
≥ 3 × 10 <sup>6</sup>	7	7	2	2	4.90	0.70–34.3
< 3 × 10 <sup>6</sup>	5	4	7	5		
All	12	11	9	7		
Non-adenocarcinoma						
≥ 3 × 10 <sup>6</sup>	0	0	5	5	NC	NC
< 3 × 10 <sup>6</sup>	3	2	20	18		
All	3	2	25	23		
All lung cancer						
≥ 3 × 10 <sup>6</sup>	7	7	7	7	3.38	0.91–12.5
< 3 × 10 <sup>6</sup>	8	6	27	23		
All	15	13	34	30		

Abbreviations: M, male; F, female; NC, not calculable.

<sup>a</sup>Logit model.<sup>b</sup>95% Confidence interval.**Table 4. Numbers of cases of adenocarcinomas and nonadenomatous lung cancer, and odds ratios for adenocarcinoma, according to asbestos exposure and presence of point mutations in codons 12 or 13 of *K-ras* in tumor tissue.<sup>a</sup>**

<i>K-ras</i> mutation	Asbestos fibers/g dried lung	
	> 3 × 10 <sup>6</sup>	< 3 × 10 <sup>6</sup>
Positive		
Number of adenocarcinomas	7	5
Number of nonadenocarcinomas	0	3
Odds ratio	41.0	4.76
95% Confidence interval	2.1-809	0.90-25.3
Negative		
Number of adenocarcinomas	2	7
Number of nonadenocarcinomas	5	20
Odds ratio	1.14	1.0
95% Confidence interval	0.18-7.28	

<sup>a</sup>Reference category: asbestos fibers ≤ 3 × 10<sup>6</sup>/g dry weight and *K-ras* negativity; logit model.**Table 5. Distribution (number of subjects) and odds ratios for point mutations in codons 12 or 13 of *K-ras* in different categories of cigarette smoking.<sup>a</sup>**

Category	<i>K-ras</i> mutation	
	Positive (n = 14)	Negative (n = 33)
Smokers		
≥ 50 pack-years	6	8
< 50 pack-years	4	15
Ex-smokers	3	8
Never-smokers	1	2
Odds ratio for ≥ 50 pack-years <sup>b</sup>	2.81	
95% Confidence interval	0.61-13.0	

<sup>a</sup>Logit model.<sup>b</sup>Reference category: < 50 pack-years (ex- and never-smokers excluded).

small amounts of asbestos play an important role in carcinogenesis in people who smoke. These inferences would be even stronger if we had a better mechanistic understanding of the possible modes of action of the two agents.

In the present study, in which lung cancer samples were obtained at surgery, we investigated the prevalence of point mutations in the *K-ras* oncogene in tumor tissue from the patients. *K-ras* mutations appeared to be

especially prevalent in adenocarcinomas, in accordance with earlier findings (6,8,14). All of the adenocarcinoma patients were either current or ex-smokers. The prevalence of *K-ras* mutations was commoner among people who smoked more (Table 5), supporting the view that tobacco smoke is an important factor in the induction of point mutations in *K-ras* in human lung adenocarcinomas. Mutations in the codon 12 of *K-ras* were mostly guanine-to-thymine transversions—base changes that can be caused, for example, by polynuclear aromatic hydrocarbons (11). For instance, in one study, benzo[*a*]pyrene-induced mouse lung tumors harbored a *K-ras* mutation in codon 12 in 80% of animals (15).

Asbestos is not an agent that causes point mutations, but it appears that exposure to asbestos increases the likelihood of *K-ras* mutations in adenocarcinomas. There may be several explanations for the increased prevalence of mutations in *K-ras* in patients with adenocarcinomas who had heavy exposure to asbestos. Asbestos fibers could increase cell proliferation and, therefore, the recruitment of mutated cells into the multistage process of carcinogenesis. This effect would be in line with the multiplicative effect observed in some epidemiological studies, suggesting that the effects of asbestos and smoking would occur at different stages. Asbestos may act by increasing gene expression and giving a selective growth advantage to mutated cells. It has been shown that people heavily exposed to asbestos have elevated levels of growth factors in their serum (10).

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